JC14 Rec'd PCT/PTO 15 DEC 2001

FORM PTO-1390 (REV. 9-2001) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: POLYMER

U S APPLICATION NO (If known, see 37 CFR 1 5): 10/018546

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/16816	16 JUNE 2000	18 JUNE 1999

TITLE OF INVENTION: APPARATUS AND METHOD FOR DETERMINING SUBSTANCES CONTAINED IN A BODY FLUID

APPLICANT(S) FOR DO/EO/US: MITCHEN et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information.

JC07 Rec'd PCT/PTO 15 DEC 2001

U.S. APPLICATION NO (if known, see 37 CFR 1.)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/018546		

21. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 710.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =		x **$18.00**	$	
Independent claims	- 3 =		x **$84.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 840.00	
☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +				$ (420.00)	
SUBTOTAL =				$ 420.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 420.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 420.00	
				Amount to be refunded:	$
				charged:	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 501306 in the amount of $ 420.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 501306. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

MICHAEL C. BARTOL
NAME

44,025
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)		
Mitchen et al.	)		
International Priority Date: 18 June 1999	)	Group:	to be assigned
International Application Number:	)	Docket:	POLYMER
PCT/US00/16816	)		
Title: Apparatus and Method for Determining	)	Examiner:	to be assigned
Substances in a Body Fluid	)		

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

The international application identified above is entering the United States of America in the national stage pursuant to 35 U.S.C. § 371. Please amend the application as follows.

In the Claims

Please cancel claims 1-6 currently pending and originally filed in the International application identified above and add new claims 1-17 as follows:

1. Apparatus for determining concentration of LDL cholesterol from a sample of whole blood, comprising:

a disbursement layer;

a blood cell separation layer in fluid communication with said disbursement layer;

a micelle layer in fluid communication with said blood cell separation layer, said micelle layer incorporated with a non-ionic surfactant and at least one

member selected from the group consisting of cyclodextrin and derivatives thereof, whereby micelles of HDL and VLDL cholesterol are formed in said micelle layer;

a hydrophobic barrier in fluid communication with said micelle layer, said hydrophobic barrier substantially trapping therein the micelles of HDL and VLDL cholesterol formed in said micelle layer; and

a reaction layer in fluid communication with said hydrophobic barrier, said reaction layer containing a cholesterol determining agent, whereby the cholesterol measurement obtained in said reaction layer substantially corresponds to the concentration of LDL cholesterol in the sample.

2. The apparatus of claim 1, wherein at least one of said cyclodextrin and derivatives thereof is selected from the group consisting of alkyl betaine derivatives, sulfobetaine derivatives, aminocarboxylic acid derivatives, imidazoline derivatives, amino oxide and ethoxylated acetylene derivatives.

3. The apparatus of claim 1, wherein said non-ionic surfactant comprises at least one compound selected from the group consisting of an aminocarboxylic acid derivative, lauric acid amidopropyl betaine, a 2-alkyl-N-carboxymethyl-N-hydroxyethyl imidazolium betaine lauryl betaine, sodium N-lauryl-N-methyl-beta-alanine and N-octyl-N,N-dimethyl-3-amminio-1-propanesulfonic acid.

4. The apparatus of claim 1, wherein said cyclodextrin and derivatives thereof is poly-beta-cyclodextrin.

5. The apparatus of claim 1, wherein at least one of said cyclodextrin and derivatives thereof is selected from the group consisting of dimethyl-alpha-cyclodextrin and poly-beta-cyclodextrin.

6. The apparatus of claim 1, wherein said hydrophobic barrier comprises an asymmetric membrane.

7. The apparatus of claim 6, wherein said hydrophobic barrier is coated with casein.

8. The apparatus of claim 1, wherein said hydrophobic barrier is coated with casein.

9. The apparatus of claim 1, wherein said hydrophobic barrier comprises a polyether sulphone membrane.

10. The apparatus of claim 1, wherein said hydrophobic barrier includes at least one compound selected from the group consisting of sorbitol, sucrose and tween 20.

11. A method of determining concentration of LDL cholesterol in a whole blood sample, said method comprising:

(a) contacting the whole blood sample with a first layer, separating blood cells from plasma in the first layer and passing the plasma therethrough;

(b) contacting the plasma which passed through the first layer with a second layer, forming micelles of HDL and VLDL but not LDL cholesterol in the second layer and passing the plasma including micelles through the second layer;

(c) contacting the plasma containing the micelles with a third layer and trapping the micelles in the third layer while passing the plasma now substantially devoid of HDL and VLDL cholesterol therethrough; and

(d) contacting the plasma now substantially devoid of HDL and VLDL cholesterol with a fourth layer that has been incorporated with a cholesterol determining agent, whereby the cholesterol measurement

obtained in the fourth layer substantially corresponds to the concentration of LDL cholesterol in the sample.

12. The method of claim 11, further comprising prior to step (a) treating the second layer with a non-ionic surfactant and at least one member selected from the group consisting of cyclodextrin and derivatives thereof.

13. The method of claim 11, further comprising prior to step (a) coating the third layer with casein.

14. The method of claim 11, further comprising prior to step (a) selecting a polyether sulphone membrane for the third layer.

15. The method of claim 11, further comprising prior to step (a) selecting an asymmetric membrane for the third layer.

16. A method of determining cholesterol concentration in a whole blood sample, said method comprising:

(a) contacting the whole blood sample with a first layer, separating blood cells from plasma in the first layer and passing the plasma therethrough; and

(b) contacting the plasma obtained in step (a) with a reaction layer incorporated with a cholesterol determining agent and CHAPS.

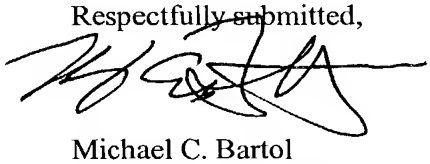
17. The method of claim 16, further comprising prior to step (b), incorporating MES buffer in the cholesterol determining agent.

Remarks

Claims 1-6 that were previously pending in the international application have been cancelled and replaced with new claims 1-17 submitted above. It is believed that this Preliminary Amendment places the claims in condition for allowance and allowance is earnestly solicited.

In the event Applicants have overlooked the need for an extension of time or payment of fee, the Applicants hereby petition therefor and authorize that any charge be made to Deposit Account No. 501306, Lowe Gray Steel & Darko, LLP If any question concerning this application should arise, the Examiner is invited to telephone the undersigned at 317/236-8020.

Respectfully submitted,



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APPARATUS AND METHOD FOR DETERMINING SUBSTANCES CONTAINED IN A BODY FLUID

BACKGROUND OF THE INVENTION

The present invention relates generally to an assay system for biological and nonbiological fluids. More particularly, the present invention relates to an apparatus for separating serum or plasma in order to measure analytes within the serum.

It has long been desirable to utilize devices that can be used for on-site testing of blood products. Particularly important is the analysis of body fluids from humans and animals to diagnose disease, monitor the course of therapy, or determine the presence of illicit drugs. Human Biological fluids may include blood, urine, saliva, feces, peritoneal fluid, synovial fluid, spinal fluid, interstitial fluid, tears, cellular fluid, pancreatic or bile fluids. Commonly, the analytical methods used to carry out these objects are performed on blood samples.

Clinical chemists have a preference for working with serum over plasma and plasma over whole blood because of the clarity of the sample matrix and the lack of interfering substances from the solid portion of the blood. In order to facilitate analysis, a separation step must be carried out since the presence of red blood cells, either intact or hemolyzed interferes with detection of the signal generated by the chemical reaction performed by the test.

Conventionally, the separation of blood components has been carried out by placing a clotted blood sample in a centrifuge and centrifuging the sample for

ten minutes at approximately 3,000 rpms. The serum obtained from this centrifuging step is then used to carry out the test, thus avoiding interference from blood solids such as red blood cells and clotting factors.

An embodiment for chemical tests called dry reagent strips was developed first for urinalysis. Thereafter, various efforts to combine dry reagent strip technology in blood testing were started in the early 1950's. Notably, U.S. Patent No. 3,092,465 discloses a reagent in a bibulous carrier with a superimposed semipermeable coating to exclude the chemical and nonchemical interference from red blood cells. The device, while performing analysis on whole blood, still required additional manipulations by the user, in the form of washing of excess blood after a specified time interval. Additionally, U.S. Patent Nos. 3,552,925 and 3,552,928 disclose the use of salts and amino acids to perform in-situ separation. U.S. Patent No. 4,477,575 discloses the use of a glass fiber matrix.

More recently, membranes have been employed in a variety of devices. These include devices disclosed in the following United States and foreign patents and publications: U.S. Patent Nos. 4,774,192 and 5,166,051; European Published Applications EP 0408222 A1, EP 0408223 A1, EP 0407800 A2 and EP 0388782; and PCT Published Applications Nos. WO 93/22453, WO 95/16207 and WO 90/10869. The use of the various membranes disclosed in the above patent documents operate on size exclusion principles, and several of these are limited by rates of capillary flow and do not completely eliminate interference from intact or hemolyzed red blood cells. Fresh red blood cells are elastic in nature and may pass through pores smaller than their nominal diameter. Hemolysis may occur on contact with some of the architectural or chemical components of

the strips. Consequently, errors may be introduced into the measurement system.

U.S. Patent No. 5,104,619 discloses a disposable diagnostic system comprising a test card having a substantially flat body and a generally cylindrical reagent pad pocket formed in a central area of the flat body. A reagent chemistry pad is disposed in the pocket and a snap fit cover is received in the pocket and arranged over the pad to retain the pad in position. The device size and configuration allows for bar code graphics to be printed on the underneath side of the device. The bar code may contain lot-specified data about the reagent chemistry, and is read by the meter during device insertion. This data may further contain critical parameters for the software algorithm within the meter electronics. U.S. Patent No. 5,139,685 also discloses a separation filter assembly having a snap fit lid. In this patent, glass fibers are utilized and maintained in a compressed state under pressure.

Accordingly, a need exists for an integrated system for assaying, in one step, analytes in whole blood samples which are not affected by the chemical or physical interference normally caused by red blood cells and other portions of whole blood.

SUMMARY OF THE INVENTION

The present invention, in one form thereof, comprises a dry solid phase diagnostic test strip and system for the chemical, enzymatic, and/or immunological analysis of whole blood analytes, comprising a reflectance photometer, a solid support strip, a porous detection zone member, a permeable

spreading and separation layer, a precipation layer and an overlay sample receiving membrane containing an agent, or agents for the exclusion of intact red blood cells and a strip receiving platform with guides for positioning the strip inside the reflectance photometer. The detection area membrane may contain chemical, enzymatic, and/or immunological reagents that generate specific signals in the presence of a target analyte. The agent, or agents, in contact with the overlay membrane, prevents passage and hemolysis of red blood cells while facilitating rapid transport and reaction of the plasma or serum portion of introduced whole blood samples. This method can be used to assay nearly every component in whole blood. For example, the lipid fractions of the blood can be individually measured. A good method was a common reagent membrane for total cholesterol determination in combination with separation layers specific to the particular fraction of interest.

In addition, the present invention, in one form thereof, comprises a reflectance photometer, which utilizes test strips that are color coded for test differentiation, thereby making complicated tests less prone to human error. For example, a blue strip may indicate a glucose test, whereas a red strip may indicate a cholesterol test. These colors are then divided into shades such as 64 shades of blue equal to 64 lot numbers of glucose strips. The photometer includes a separate optical read head that determines the color and shade of the base of the test strip device as the strip is inserted into the photometric instrument. The shade is converted into a lot number ranging from 1 to 64. The instrument also has a memory module (preferably an electrically erasable programmable read-only memory) that has a corresponding lot number to the

shade of the strip to ensure lot number verification. The instrument then compares the inserted memory module programmed lot number to ensure that it is the same lot number as the test strip. If the strip lot number does not match the memory module lot number, the test is not performed, and the user is instructed to insert the correct memory module.

The lot number verification allows for the automated coding of lot numbers so that the user does not need to enter a lot code for each vial of strips. This prevents the running of the incorrect, old, or expired lot number tests in the instrument.

The "plug-in-memory" of the module includes the lot number of the test strip, the expiration date, and the performance criteria for the actual strip measurement. The performance criteria include the wavelength, measurement algorithm, and unreacted density qualifications necessary for a valid test result.

The optoelectronic measurements of the chemistry test reaction on and in a surface enhances the dynamic range of the dry phase test. Algorithms that read at different wavelengths at different times in the chemistry reaction can extend the dynamic range of the test system. This is particularly applicable when using multiple chromophores in a single measurement system. The early portion of a chemistry could be read at the peak wavelength of a reaction, while the later portion, or darker or more dense portion of color development could be read at a wavelength not near the peak of the color development. In addition, different chromophores may respond in a more linear manner in different portions of the dynamic range of the chemistry. Manipulation of these two data points can significantly increase the dynamic range (in mg/dl) of a chemistry reaction.

The optoelectronic measurement of the chemistry test reaction on and in a surface reduces errors due to orientation of the surface to be read to the instrument. Multiple wavelengths and different angles are used to correct possible problems in positioning the strip in the instrument. If the detector is at "0" angle and the emitters of the same or different wavelengths are at different angles (e.g., one at 40° and one at 50°), the tilting of a surface will positively contribute to one reading while it will contribute in a negative manner to the other reading, thus it is able to cancel the error presented by the angle presentation of the surface. These same measurement methods can be used to eliminate interferences from substances such as billirubin and others.

The optoelectronic measurements of the chemistry test reaction on and in the surface enhance the stability of timed and untimed dry phase chemistry reaction. Algorithms are used to determine the "end point" of a chemistry. In other words, measurements can be done at similar or dissimilar wavelengths to predict the stable portion or end point of a chemistry if kinetic measurements are made, the kinetic readings can be subjected to an algorithm to determine that the rate is slow enough to declare the extrapolate chemistry is at an end or completion. When known standards are run and predicted by this pseudo-endpoint, the same measuring criteria can be applied to unknowns to determine the "endpoint' of the test reaction.

The use of colored or shaded visual indicators in the instrument enhance the interpretation of test results. A colored bar graph is used to aid the user in knowing when the user test results are in a normal or safe range. Out of range colors such as orange for caution and red for danger are used when results are outside the green "safe" range. This is particularly useful to new testers who are

not familiar with the number scale of the different test results. A voice module can also be used to warn the user of unsafe results or operation of the instrument system to make the system usable by the visually impaired by providing, for example, a sound beep for each unit of glucose during a glucose test.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of the reflectance photometer in accordance with an embodiment of the present invention;

Fig 2 is an exploded perspective view of the plastic test strip of present invention in its unlocked position;

Fig 3 is a perspective view of the plastic strip of Fig. 2 in its locked position; and

Fig 4 is a sectional view of the plastic strip;

Fig. 5 is a block diagram schematic of one embodiment of the reflectance photometer of the present invention;

Fig. 6 is a graph plotting sample size, elapsed test time and percentage of reflectance illustrating how endpoint determinations may be utilized to speed chemistry measurement.

BRIEF DESCRIPTION OF THE PREFERRED EMBODIMENT

In accordance with the embodiment of the present invention, the diagnostic chemistry measurement device 10 for dry solid phase chemical, enzymatic, immunological assay of whole blood or sera analytes is made up of an injection molded carrier test strip 20 in which several porous and nonporous materials containing chemicals and reactants are contained for the purpose of generating a detectable signal in the presence of certain analytes. The test strip

12 is inserted into a reflectance photometer. The reaction material layer and the test strip 12 is held in intimate noncompressed contact with a whole blood separation layer in the absence of adhesives for the purpose of providing a liquid sample free of red blood cells to the reaction layer or layers.

HOLDER

The holder test strip 12 of this invention acts as holder for the different layers of the test reaction system. It provides a convenient handle as well as a mechanism for placing test strip 12 into an instrument 10 for the reading of the density changes of the reaction layers. As shown in Fig. 2 test strip 12 includes an elongate body 16 preferably formed by injection molding . Elongated body 16 includes a first end portion 18 and a second end portion 20. A hinged portion 22 is located between first and second and end portions 18 and 20 so that first end 18 is foldable over elongated body 16 into contact with second end 20.

As shown in Fig. 2 first end portion 18 includes an opening 24 while second end portion 20 includes a complementary spaced opening 26. When first end portion 18 is folded over body 16, each opening 24 and 26 are aligned. In its folded position as shown in Fig. 3 opening 24 in test strip 12 defines an area for depositing a body fluid sample while opening 26 defines an area in which optoelectronic measurements of chemistry test reactions are conducted.

Test strip 12 further includes a non-adhesive carrier layer 14 formed from, for example, five particular layers. In a standard diagnostic test strip, carrier layer 14 may include a disbursement layer 28, formed of, for example, woven materials such as polyester or cotton, for rapid and even disbursement of body fluid along carrier layer 14. Beneath that may be included a separating binding and/or

precipitating layer 30, constructed of known materials such as shown in Table IX infra, that, when exposed to a sample liquid, may separate analyte and analyte disrupting elements such as red blood cells from whole blood. This action would permit the serum analytes to pass through separating layer 30a and 30b while preventing red blood cells or other analyte disrupting elements from passing through. The last layer shown in Fig. 2 is that of the test reaction membrane 32 on which the dry chemicals and reactants are contained for generating a visible signal, in the presence serum analytes. Molded carrier body 16 serves as a support for the reacting and non-reacting layers 28, 30 and 32 which may be formed from papers, membranes and deles materials.

The test strip holder 12 positions the different layer materials 28, 31, 32 within the holder the correct X, Y and Z axis positions. Carrier layer 14 made up, for example, the disbursement separating and test reaction layers 28, 30 and 32 are held in noncompressed non-adhesive locations by first end portion 18 folding over to second end portion 20. This may be accomplished in a number of different ways. The preferred way of noncompressingly holding carrier layer is of an upstanding annular rim 34 may help locate the carrier layer 14 within test strip 12. Additionally, small upstanding protuberances 36 along second end portion 20 radialy located away from opening 26 prevent movement of carrier layer 14. The purpose of both annular rim 34 and small upstanding protuberances 36 is to hold the layers of carrier layer 14 without compression between opening 24 and opening 26, hereby preventing pooling of any sample within carrier layer 14. This consideration of noncompression of the carrier layer 14 is of greater importance when larger numbers of layers are utilized. The positioning of a carrier layer 14 without adhesives or compression allows for efficient transport of sample and

reactants contained in the system and test strip 12. Annular rim 34 or alternatively other areas of test strip 12 may include sawtooth protrusions to increase flow rate thorough carrier layer 14.

Test strip 12 includes a locking mechanism to prevent any unlocking of front-end portion 18 from its folded position over elongated body 16. As shown in Fig. 2, one type of locking mechanism may include a plurality of upwardly extending tabs or projections 38 that interfit or lock into corresponding openings 40 in first end portion 18. When first end portion 18 is folded to second end portion 20, lock projections 38 will interfit and snap lock with openings 40. Other types of one way locking mechanisms may also be used such as snap rivets.

More than one test reaction system can be housed in a test strip 12. A second set of holes 24, and 26 may be included in test strip 12 so that two tests may be run at once.

The described holding mechanism allows for the rapid separation of whole blood into its liquid and solid components. It also allows sample volumes as low 2.0 microliters to be used in dry phase chemistry reactions. Test strip 12 allows the use of several reaction and non-reaction layers. A typical holder could contain from 1 to 8 layers of material with thicknesses from approximately 0.002 inches to 0.007 inches, for example.

Chemicals and materials are employed to allow for the treatment of samples such as whole blood, which will allow the whole blood sample to be separated without disrupting the red blood cells while rapidly moving the liquid portion of the whole blood sample to one or more reaction sites in the holder, normally on a test reaction membrane 32. These chemicals can be composed of polymeric and nonpolymeric substances that are dried onto one or more surfaces of the

materials contained in the device holder. Additionally, light metal salts of elements such as Potassium, Lithium, Sodium, and Calcium may be utilized to treat red blood cells before and during the separation process. The materials which may be used in the holder for treatment by or containment of these chemicals can be composed of woven, nonwoven, napped or flocked materials.

LDL

The present invention includes a homogeneous dry phase assay for LDL Cholesterol that can replace assays that previously required more than one operation to achieve the desired measurement. Enzymes are used to digest HDL Cholesterol. This method uses a chemical complexing layer (ex. Surfynol 465, Surfynol 1485, Silwet L 7600, nDodecyl-B-D maltoside, Polyoxyethyglene ether, Sucrosenomocaphate. A hydrophobic barrier does not allow the complex formed in #2 to react with the cholesterol reaction membrane. The best example of this is a Polyether sulphane asymmetric, treated with casein, to make it hydrophobic. Casein coated membrane will not function alone because of its hydrophobicity: this property is adjusted by the addition of sorbitol and other agents, such as sucrose or tween 20. In comparison to PCT #WO95/16207, a barrier to control flow through the device is used, in contrast to the devices described in this publication. This device does not have an absorbent or wick at far end of device, and this is not a chromatographic device. It was found that when the flow of liquid into the capillaries of a dry device encounters a restrictive barrier, the change in pressure due to this resistance can accelerate in one direction only until the device (the final reaction membrane) is wetted by the sample.

Thus, the sample flows in one direction in a more rapid rate than any other direction. This unidirectional flow allows for the chromogen and other reactants to move in one direction only without the use of an absorbent as in W095/16207. Thus, the flow rate is controlled by the use of multiple holes, dissimilar in size, (not unlike adjusting a hose sprayer nozzle.) Examples include: aperture sizes for aperture A, 0.050-0.250 and aperture B, 0.2-2.0 microns, and for Aperture C, whatever test strip holder size is. The apertures control unidirectional flow without using a proximal (final) absorbent layer (wick). Other devices with apertures contain a wick downstream. This construct is also used for HDL.

HDL

The present invention includes a single reaction membrane with low holdup volume for the precipitation and/or binding of VLDL and LDL. This makes it possible to rapidly detect HDL in one step using whole blood. This direct flow-through device is different than devices from BMC, Kingston and Cholestech that are all tangential flow systems.

Use of CHAPS in place of cholate in cholesterol reagent

CHAPS, or 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate, is made by Research Organics of Cleveland, Ohio, for use in cholesterol reagents because of problems people were having with cholate, the usual surfactant is diagnostic cholesterol reagents. The following are some advantages of using CHAPS in place of cholate in the reagent:

1. CHAPS is more soluble and also more easily reconstituted by plasma than is cholate, thus it can more readily enhance pancreatic esterase activity than cholate.
2. As a result of the increased pancreatic esterase activity there is more chromogen production and thus the resolution and dynamic range of the test is increased.
3. Since CHAPS is more readily resolubilized than cholate, the diagnostic test strips will have greater precision with CHAPS because it will be maximally and more uniformly distributed throughout the reaction membrane immediately after the addition of blood to the strip.
4. Because of its different properties, CHAPS was found to be more compatible with a larger number of different chromogenic systems, particularly the Trinder systems than was cholate. In solutions made with cholate, 4-aminoantipyrine could not remain in solution.
5. Since CHAPS is a more acidic surfactant than cholate, it is able to go into and remain in solution at a lower pH than can cholate. This allows for lowering the pH of its reagent from 6.8 to 6.0 and thus eliminating uric acid interference.

Use of MES buffer in cholesterol reagent

When MES, or 2-[N-morpholino]ethanesulfonic acid, was used as a buffer in cholesterol reagent in place of citrate, it was found to:

1. help solubilize and stabilize (keep in solution) certain cholesterol esterases necessary for the optimal performance of the assay.
2. Increase the amount of color generated per mg/dL of cholesterol thus improving the test's precision. This is probably the result of better performance by the esterase(s).

Substitution of Rhodasurf ON-870 for Triton X-100 in a strip based D-3-hydroxybutyric acid assay

When Rhodasurf ON-870 (a mixture of ethoxylated tridecanol, ethoxylated tridecanol phosphate ester, and phosphoric acid), or a similarly formulated surfactant, was used in place of Triton X-100 (t-Octyphenoxypolethoxyethanol), or a similarly formulated surfactant, in a dry, strip based D-3-hydroxybutyric acid assay the following benefits resulted:

More ready solubilization and hence reconstitution of the tetrazolium indicator resulting in:

1. Increased precision due to more uniform dispersion of regents after reconstitution with whole blood;
2. More rapid kinetics, resulting in the average test time dropping from 120" to 40"; and
3. Increased dynamic range; from 0-10 mg/dL to 0-60 mg/dL D-3-hydroxybutyric acid.

Enhanced stability of the D-3-hydroxybutyrate dehydrogenase.

The first benefit, as well as the three numbered entries that it subsumes, is probably applicable to a number of assays using tetrazolium salts as a chromogen, especially in a dry format.

ANALYTES

A wide variety of analytes can be determined by using the disclosed apparatus. Examples are given in tables I and II, infra.

Further, given the small size and robust nature of the reagent strips and instrumentation, analyses need not be limited to traditional clinical laboratory settings. The device of the present invention is also simple enough to be used by people with minimal or no chemical or medical technology training. This advantage allows use at home, or by mobile health are delivery people. Examples of this are diabetics who must monitor themselves for glucose and ketone bodies, patients on home dialysis who would benefit by monitoring of urea nitrogen and people endeavoring to lower their cholesterol levels.

Further, by combining several different reagents on a single support, a panel of tests may be done. Examples of this would be a liver panel consisting of ALT, AST, Alkaline Phosphates. A diabetic panel might consist of glucose, beta hydroxybutryrate and glycated hemoglobin. A coagulation panel might consist of Prothrombin time, ACTT and ACT.

FAMILIES OF ANALYTES BY STRUCTURE

Table 1

Family	Examples
Carbohydrate	Glucose, lactose, galactose
Nitrogen Moiety	Urea nitrogen, creatnine, uric acid
Lipid	Cholesterol, triglycerides, LDL, HDL
Enzyme	ALT, AST, Alkaline Phosphatase, CPK, CK-MB
Hormone	Hcg, lh
Therapeutic Drugs	Theophylline
Drugs of abuse	Cocaine, marijuana, barbiturates, salicylates
Electrolyte	Na + K + C1 Li + CO_2

Nucleic Acids	Infectious disease, forensic applications, genetic disorders

FAMILIES OF ANALYTES BY DISEASE

Table II

Disease	Examples
Diabetes	Glucose, beta hydroxybutyrate, hemoglobin A_{1c}
Liver problems	ALT, AST, bilirubin
Acidosis/Alkalosi	PO_2, $PCO^2$1 pH
Hypertension	Na+, K+
Nutritional status	Ca, Mg Zn trace minerals

Examples

The following illustrative examples teach various combinations of buffers, dyes, stabilizers and other reactive and functional components which may be combined by a person having ordinary skill in the art into the system test reaction areas.

Table IX gives various types of dyes and indicators used in diagnostic reagents.

Example #1

Glucose measuring system

Table III

Ingredient	Function	Amount	Available from:
Glucose Oxidase	Reactant	25,000	Sigma Chemicals

			St Louis, MO
Peroxidase	Reactant	75,000	Sigma Chemicals St. Louis, MO
Silwet 7500	Serfactant	0.10 ml	Dow-Corning Midland, MI
PVP K 30	Enzyme Stabilizer	0.50 gms	ISP Linden, JN
Citric Acid	Buffer System	1.25 gms	Aldrich Chemical Milwaukee, WI
Sodium citrate	Buffer System	0.10 ml	Dow-Corning Midland, MI
DOW 1520	Antifoam	1.00 gms	Aldrich Chemical Milwaukee, WI
4 AAP	Chromophore	0.25 gms	Aldrich Chemical Milwaukee, WI
3 5 DCHBS	Chromophore	0.25 mgs	Boehringer Mannheim
Distilled H_2O	Solvent	QS to 100 ml	

Preparation: Approximately 50 ml of distilled H_2O was placed in a beaker on a stirring plate. A magnetic bar was added and the ingredients added sequentially after the previous gradient was dissolved and dispersed. After all ingredients were added the volume was adjusted to 100 ml of distilled H_2O .

Example #2

Triglycerides measuring system

TRIGLYCERIDES + H_2O $^{CHOLESETEROL\ ESTERASE}$ GLYCEROL + FREE FATTY ACIDS GLYCEROL + ATP $^{GLYCEROL\ KINASE}$ L – ALPHA – GLYCEROPHOSPHATE + H_2O_2 H_2O^2 + 4-AMINOANTIPYRINE + DCHBS ^{PEROXIDASE} QUINONEIMINE CHROMOPHORE

Table IV

Ingredient	Function	Amount	Available from
Cholesterol esterase	Reactant	15,000 units	Shinko-American NY, NY
Glycerol kinase	Reactant	5,000 units	Shinko-American NY, NY
Glycerophosphate Oxidase	Reactant	5,000 units	Shinko-American NY, NY
Peroxidase	Reactant	5,000 units	Shinko-American NY, NY
4 AAP	Chromogen	1.0 ogm	Aldrich
3, 5 DCHBS	Chromogen	0.25 gm	Boehringer Mannheim
MES	Buffer	2.50 gm	Research Organics
PVP K30	Stabilizer	0.50 gm	ISP
Glucose	Filler	2.50 gm	Sigma
Triton X-100	Surfacant	0.10 gm	Boehringer Mannheim
Distilled H_2O	Solvent	QS to 100 ml	

Preparation: Same as example #1

Example 3

Cholesterol measuring system (all amounts approximate)

Table V

Ingredient	Function	Amount	Available from
Choleseterol oxidase	Reactant	10,000	Shinko-American NY, NY
Cholesterol esterase	Reactant	7,000	Shinko-American NY, NY
Sodium phosphate 0.5 M pH 7.0	Buffer	750 ml	Dow-Corning
BSA	Surfacant	15 gm	Aldrich Chemical
Peroxidase	Reactant	170,000	Shinko-American NY, NY
DOSS	Surfactant	7.0 gms	Boehringer Mannheim
Sucrose	Stabilizer	1.0 gms	Sigma Chemicals
TMB	Chromogen	10.0 gms	Aldrich Chemical
Distilled H_2O	Solvent	QS to 100 ml	

Preparation: Same as example #1

Alternatively, the chromogen may be prepared in an organic solvent matrix and treated as a first or second application to the membrane or paper.

Table VI

Ingredient	Function	Amount	Available from
Acetone/methanol	Solvent	100 ml	Aldrich

1:1			
Tetramethyl benezidine	Solvent	1.0 ogm	Biosynth Inc Chicago, IL

Example 4

Blood Urea Nitrogen Measuring System

Table VII

Ingredient	Function
Urease	Reactant
H_2O	Solvent
Bcomthymol blue	Chromogen
PVP K90	Film former
Fructose	filler

Preparation: Same as experiment #1

Table VII

Types of Indicators

Chromogenic substrate

Redox

Leuco dyes

Oxidative couplers

Benzidene Derivatives

Fluorescent labels

Dye releasing system

Table IX

Separation mechanisms used in dry reagents

Chemical	Physical	Mechanical
Dextran	Hydrophilic polymers	Centrifuge
Sugars	Porous latex films	Filters
Lectin	Polymer & swelling agent	Filters & pressure
Amino acids PEG/polyacrylate	Membranes microfiber cloth	Membranes & differential pressure wedge shape
Thrombin	Napped cloth	Gels
Sintered porous matrix	Coagulants	Density gradient
Agglutinating agents	Glass fibers	
Amine polymers	Hollow fibers	
Trivalent cations	membrane	

Example 5

HDL Formulation

Table X

Chemical	Quantity/100ml
DI WATER	80 g
PTA	1.70 g
Mg Sulfate	0.75 g
Sodium EDTA	0.10 g
DiPotassium Phosphate	1.21 g
QS to 100 ml	

Example 6

LDL Formulation

Micell layer (i.e, Michele layer) S&S 595 coated with Surfynol (.9%); Alpha-Cyclodextrin (1.5%) Tween 20 (0.05%); BSA (1%); NaCL (1%); d-Sorbitol (1%). Separation Layer, BTS#45, BTS#5, or Millipore PES tight side down, coated with Casein (Pierce Blocking Solution) and 1%D-Sorbitol. Standard Cholesterol Membrane, Biodyne A.

SPECTROPHOTOMETER

The present invention also includes use of a spectrophotometric device 10 for determining the density of the color reaction on and in the membrane surface of the test reaction layer 32 within test strip 12. Photometric device 10 as shown in Fig. 1 includes a hand-held housing 50 for containing electronic control circuitry for operation the aforementioned tests. In the embodiment shown in Fig. 1, a test strip holding region 52 is located above three light detectors or sensors 54 each disposed within a port 56. During test operation, a test strip 12 is inserted into holding region 52 so that test strip openings 26 are located adjacent ports 56. Light sensors may take a reading from light reflected from the exposed test reaction membrane layer 32 or from test strip 12 itself to determine its color.

Housing 50 further includes a specialized display device, such as a liquid crystal display 58. Display 58 is utilized for relating test results and other information to the user. In particular, a color scale 60 is used to facilitate interpretation of test results operating concurrently with digital display segments 62. Additional display segments on display 58 include a test wait indicator segment 64 to inform the user to wait while device 10 is performing the selected

tests, and a test name segment 66 which the unit determined from the type of test strip 12 inserted.

Color scale 60 may easily be constructed by a plurality of shaded or colored segments arranged adjacent each other to form a bar graph like indicator. Electrically controllable segments 68 are oriented over the color or shaded segments so that when segments 68 are activated segments 68 become dark, preventing certain colored or shaded segments 60 from being visualized or viewed. Segments 68 that are not activated permit the underlying colored or shaded segments of color scale 60 to be visualized. In this way it is possible for an electronic control to permit only a single colored or shaded segment to be viewed thereby communicating test results.

A possible result range spectrum for color scale indication segments may include particular colors with particular test result meanings such as:

Very high result danger, RED

High result danger, RED

High result caution, YELLOW

High result caution, YELLOW

High normal result, GREEN

Normal result, GREEN

Normal result, GREEN

Low normal result, GREEN

Low result caution, YELLOW

Low result caution, YELLOW

Very low result danger, RED

Color scale 60 permits an unsophisticated user to instantly visually determine, in one embodiment, if a test result is normal (a green segment visualized), slightly abnormal (a yellow segment visualized) or dangerous high or low result (a red segment visualized). Alternatively, if a color liquid crystal display is utilized, the electronic control for test unit 10 may directly indicate a colored segment, rather than covering all but one colored segment.

A suitable instrument, such as a diffuse reflectance spectrophotometer 10 with appropriate software, can be made to automatically read reflectance at certain points in time, calculate the rate of reflectance change, and by using calibration factors and software, output the level of analyte in the fluid tested. The electronic control mechanism of photometric unit 10 is shown in schematic form in Fig. 5. One or more light sources 70, for example high intensity light emitting diodes (LED) are disposed in housing 50 to illuminate test strip 12 as shown by arrows 72. A light detector or sensor 54 can be adapted to generate or respond to particular wavelengths of light.

Sensor 70 transmits a signal to an amplifier 74 as is known in the art. One type of amplifier available for use is, for example, a linear integrated circuit, which converts the phototransistor current to a voltage signal.

Appropriate electronic circuitry is utilized to take the output of amplifier 74, normally a sample and hold unit 76, and transfer the signal to an analog-to-digital converter78. Analog-to-digital converter takes the analog voltage output from the sample and hold unit 76 and converts it to, for example, a 16 bit binary digital

number upon command of a microprocessor/microcontroller unit 80. Preferably an electronic microprocessor/microcontroller 80 utilizing digital integrated circuitry is used to time selected tests, read signals and together with associated programs and data memory 82, calculate and store reflectivity valves and calculate analyte levels from the stored data.

Additional information for particular tests may be stored in a removable EEPROM unit 84 operably connected to microprocessor/microcontroller 80. EEPROM unit 84 is an interchangeable plug-in memory module containing measurement parameters, software, calibration data, and reagent recognition data for particular test strips 12. Additionally, EEPROM unit 84 contains the shelf life data and identity verification information for particular production runs or lots of test strips 12.

Automated lot coding is done by color coding the plastic material used to make the test strip holder 12. The color used in test strip holder 12 preferably has 16 different densities that can be distinguished by at least one of the wavelengths used in the optical sensor head 54 of instrument 1.

As the strips 12 are inserted into device 10, the instrument detects a change in the measurement area. This change indicates that a strip 12 has been inserted into the instrument 10. As the instrument detects the insertion of a test strip 12, it reads the densities of at least one of the LED's and calculates the lot number by the above table. Instrument 10 then goes to the EEPROM port connected to microprocessor/microcontroller 80 which has an EEPROM unit 84 inserted. Instrument 10 checks to see that the EEPROM preselected lot number is the same as lot number of test strip 12 that had been inserted into the

instrument. If the lot numbers are the same for test strip 12 and EEPROM 84, the instrument downloads the information contained in the EEPROM and proceeds with the test analysis. The instrument 10 reads the density of the unreacted strip to assure quality of the strip before the test is initiated, if quality is passed then the instrument instructs the user to apply a sample.

A sample is then applied and instrument 10 begins a measurement cycle to ensure that the proper amount of sample was applied to the test strip. When the instrument has determined that enough sample has been applied, it then goes into another cycle to measure the end of the chemistry reaction. When the end of the chemistry reaction has occurred, then the instrument measures the final density and compares it to a measurement algorithm stored in EEPROM unit 84. This measurement algorithm then determines the concentration of the test to be measured by comparing the measured density (darkness) of the color formed and comparing this density number to a table of values through the use of an algorithm stored in the EEPROM unit 84.

After a particular test strip is selected and placed in the unit, a sample normally a whole blood sample from a fingertip or from a pipiter tip (which could have gotten its sample from a tube of blood as in a laboratory type situation), is applied to the sample application spot, opening 24, on test strip 12. A disbursement layer 28 causes the sample to quickly spread over the entire area of carrier layer 14. The separation layer 30 of the test strip spot is allowed to separate out the solids (red blood cells and other analytes disrupting elements) from the liquid (plasma or sera or other analyte containing portion). The separated fluid, i.e., the plasma, sera, or other analyte containing portions, moves to the test reaction membrane layer 32 below the separation membrane 30. The

above fluid migration causes the reactants (analytes such as glucose) in the sample to come into contact with the reactants in test reaction membrane layer 32.

Analytes/fluid contacts reagent layer reaction 32 and initiates an appearance or disappearance of color, depending on its particular reaction. The above presentation of analyte to the reaction layer 32 causes the desired reaction to occur. This reaction causes a color change that can be detected both visually and by the instrument. The color change is then converted into a digital result on the instrument LCD as described above. A comparison color chart can be used to visually determine a reaction quantity scale as in litmus paper.

Instrument 10 can use different wavelengths at different density portions of the reaction to maximize the dynamic range of the chemistry and the limits of the instrument at a particular wavelength.

The "end-point" of the reaction is defined as a point where there appears to be no change or a very small change in density. That is, the chemistry changes color proportional to the concentration of the reactance that has come into contact with the reactance materials in the test pad (membrane). This small amount of change can be a change per time period. An example would be as per the graph in Fig. 6. Detailed information used to generate this graph is that the changes per 5-second time period during the beginning of the test reaction would be greater than 5% reflectance per 5-second time period. When this change is less than 1% reflectance per time period it can be said that the reaction is complete or at an endpoint. The instrument stores this percentage reflectance at this time and uses it as above to determine the concentration of the analyte tested for in the test strip.

The Kubelka-Monk equation of K/S=(1-reflectance)^2 divided by (2 x reflectance) can be used to linearize the percentage reflectance values. This linearization simplifies the algorithm necessary to calculate results. This pseudo endpoint chemistry allows a more stable read time, which in turn allows for a more reproducible answer. Pseudo endpoints also permit a more rapid assay to be performed. Certain other glucose monitoring systems incorporate pre-determined timing circuit. This pseudo endpoint allows for a different method to be used in measuring chemistry reactions, provided one can determine the endpoint of the chemistry by a method other than timing.

Multiple wavelengths are used to enhance the dynamic range of chemistry. This is particularly useful when one uses a multiple chromophore indicator system as do some of the above mentioned chemistries. Early portions or low concentrations of a test such as glucose can use a broad range indicator such as TMB to increase sensitivity in the low to mid range of the chemistry. When the test concentration is higher or the reaction faster, a different chromophore is focused upon to determine more dynamic range than the previous chromophore. This allows one to expand the dynamic range by two different methods.

One can also use wavelengths on the peak for more dynamic range and wavelengths off the "peak" absorbence of the test system to enhance or reduce dynamic range and also to enhance or reduce the ""pseudo endpoint" algorithms. Manipulation of these four factors, chromophore A, chromophore B, wavelength 1 and wavelength 2 can allow one to better define the "pseudo endpoint" algorithm and also allow one to optimize the dynamic range of the chemistry which in turn

allows for increased sensitivity throughout the chemistry reaction range with greater precision.

Multiple wavelengths can also be sued with different angles of emission of correct possible problems in positioning the strip in the instrument. If the detector is a "0" angle and the emitters of the same or different wavelengths are at different angles (one at 40° and one at 50°) the tilting of a surface will positively contribute to one reading while the other contributes in a negative manner, thus canceling the error presented by the angle presentation of the surface. These same measurement methods can be used to eliminate interference from substances such as bilirubin and others. When the angle of light incidence is increased from improper positioning of a chemistry read surface to the instrument optics, errors of both gloss and angularity are introduced into the measuring system and can give false low readings.

Examples

1. Indicators and chromogens advantageously used in combination
   a. wide range pH test

      Bromothymol blue and methyl red covers pH range of 5 through 9
   b. 4 amino antipyrine + 3.5 dichlorohydroxybenzene sulfonate (4AAP _3.5 DCHBS)
   c. TMB + Chromotropic acid
   d. Syringaldazine _ Vanillin Azine
2. Color coding for test and lot identification
   a. Blues, 16 different shades (density)
   b. Reds, 16 different shades (density)

c. Greens, 16 different shades (density)

d. Yellows, 16 different shades (density)

e. Oranges, 16 different shades (density)

f. Browns, 16 different shades (density)

g. Magentas, 16 different shades (density)

h. Light blues, 16 different shades (density)

i. Light reds, 16 different shades (density)

j. Light greens, 16 different shades (density)

k. Light browns, 16 different shades (density)

l. Light magentas, 16 different shades (density)

m. Cyan, 16 different shades (density)

n. Light cyan, 16 different shades (density)

It will be appreciated that the foregoing is presented by way of illustration only, and not by way of any limitation, and that various alternatives and modifications may be made to the illustrated embodiment without departing from the spirit and scope of the invention.

What is claimed is:

1. A process for measuring cholesterol in low density lipoproteins present in a living sample by optically measuring a reaction product of the living sample with a reagent, which comprises conducting the reaction of the living sample in the presence of an non-ionic surfactant and at least one member selected from the group consisting of cyclodextrin and derivatives thereof.
2. The process according to claim 1, wherein the cyclodextrin derivatives is at least one compound selected from the group consisting of dimethyl-alpha-cyclodextrin and poly-beta-cyclodextrin.
3. The process according to claim 1, wherein the cyclodextrin derivatives is poly-beta-cyclodextrin.
4. The process according to claim 1, wherein the amphoteric surfactant is at least one compound selected from the group consisting of alkyl betaine derivatives, imidazolinium betaine derivatives, sulfobetaine derivatives, aminocarboxylic acid derivatives, imidazonline derivatives, amine oxide and ethoxylated acetylene derivatives.
5. The process according to claim 1, wherein the amphoteric surfactant is at least one compound selected from the group consisting of an aminocarboxylic acid derivative, lauric acid amidopropyl betaine, a 2-alkyl-N-carboxymethl-N-hydroxyethl imidazolinium betaine lauryl betaine, sodium N-laurol-N-methyl-beta-alanine and N-octyl-N, N-dimethyl-3-amminio-1 propanesulfonic acid.
6. A process for measuring cholesterol in low density lipoproteins in a living sample, which comprises: treating the living sample with a first reagent comprising at least one member selected from the group consisting of

cyclodextrin and derivatives thereof along with a suitable surfactant; measuring reflectance resulting in color on a membrane reactive to cholesterol; containing cholesterol oxidase, cholesterol esterase, and peroxidase with electron acceptors which change colors; and providing the cholesterol amount in the living sample on the basis of the reflectance data measured above, wherein a coupler, a developer, peroxidase, a surfactant and cholesterol esterase are contained in at least one or two layers.

ABSTRACT OF THE DISCLOSURE

An apparatus for the optoelectronic evaluation of test paper strips for use in the detection of certain analytes in blood or other body fluids. The test strip comprises an elongated plastic part including a hinged portion to allow a first portion to be folded-over a second portion during assembly. A series of layers of test strips are disposed between the folded over portions of the test strip. The test strip is configured such that the chemistry layers are placed in contacting engagement with one another, but not compressing one another. A series of holes or apertures controls the flow-through without using an absorbent layer at the end (wick). A reflectance photometer is provided and includes various features, including a lot number reader wherein if the test strip does not match the memory module, a test is not performed, and the user is instructed to insert a correct memory module.

Other features include methods for determining cholesterol, HDL, LDL using novel techniques.

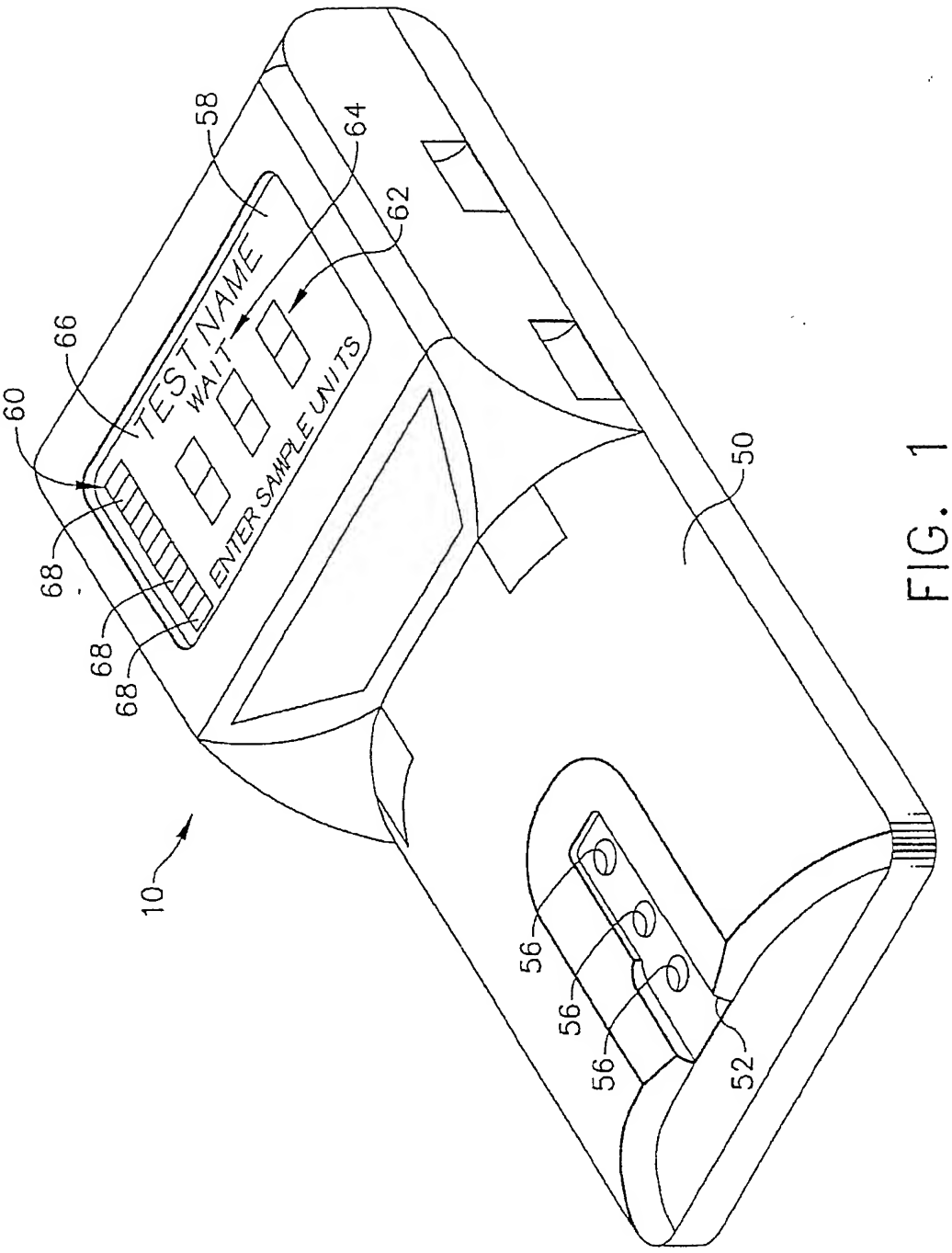
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FIG. 1

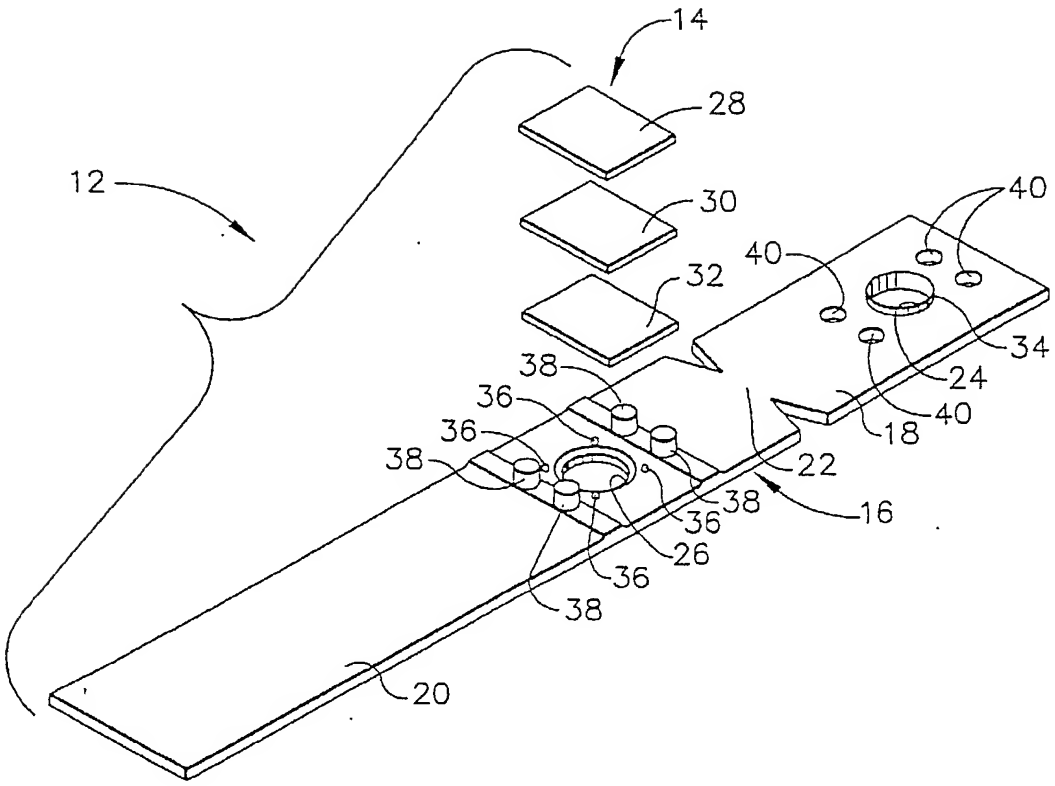
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FIG. 2

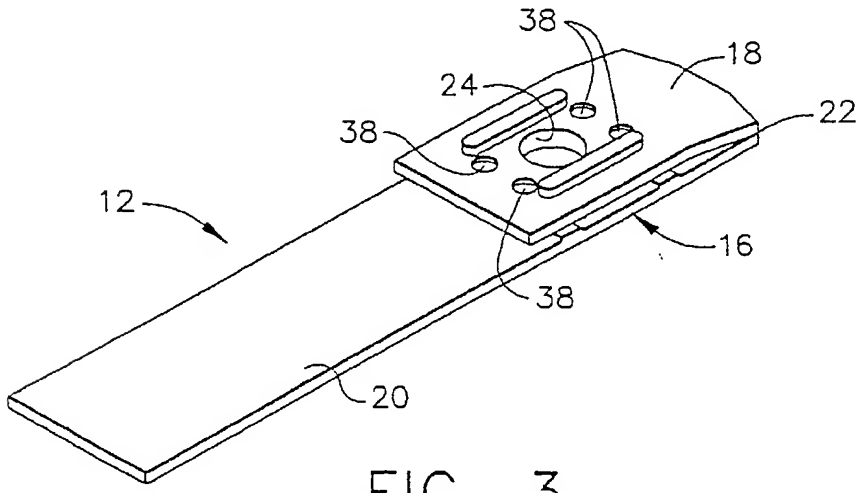
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FIG. 3

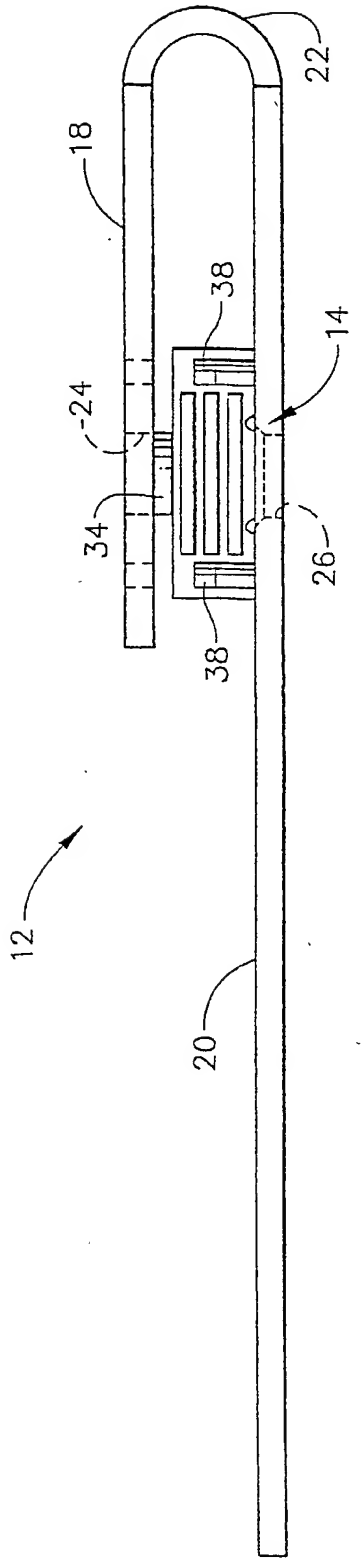
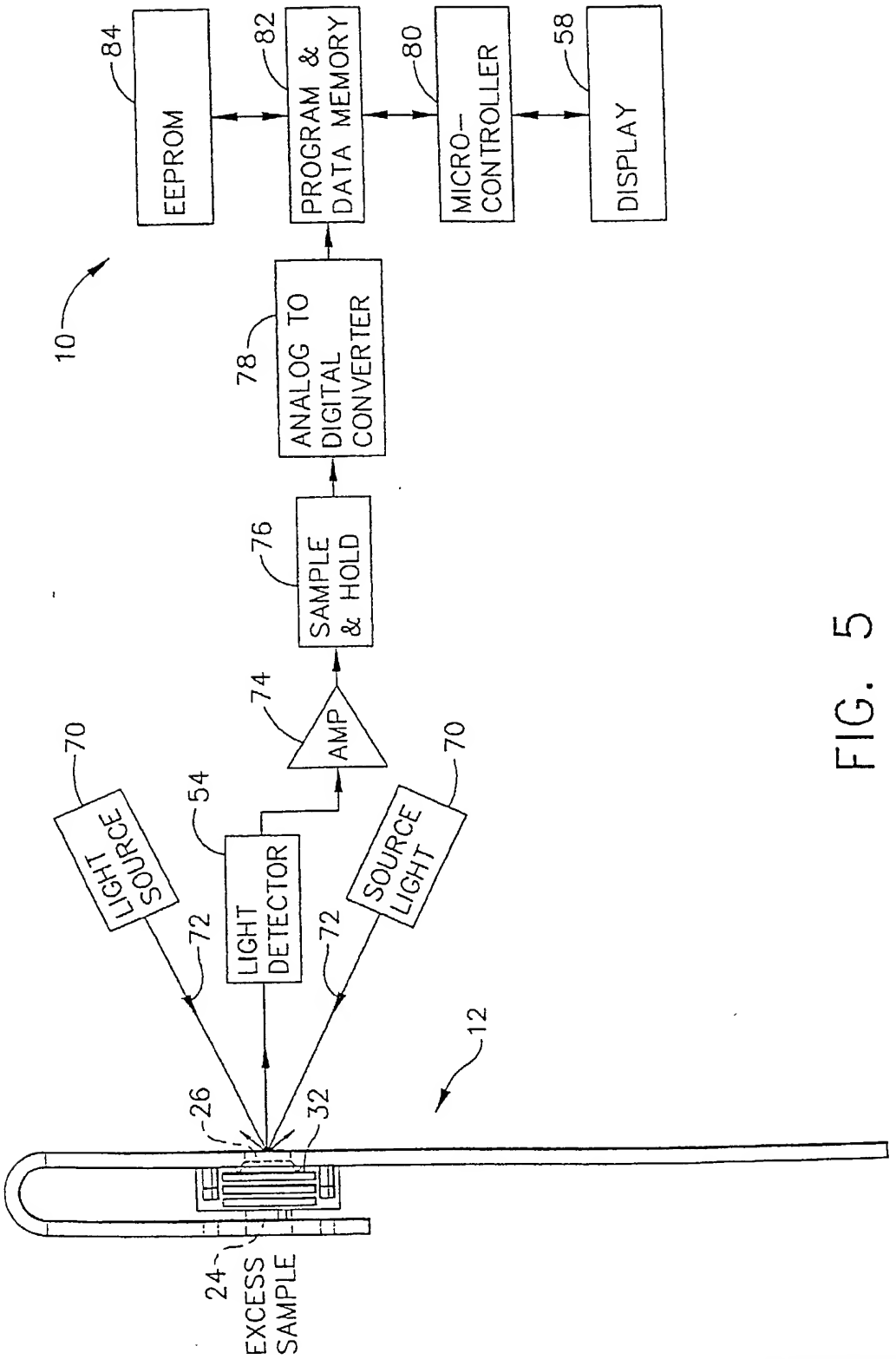


FIG. 4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MICRO-CONTROLLER
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DISPLAY
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ANALOG TO DIGITAL CONVERTER
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LIGHT DETECTOR
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SOURCE LIGHT
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32
24
EXCESS SAMPLE

FIG. 5

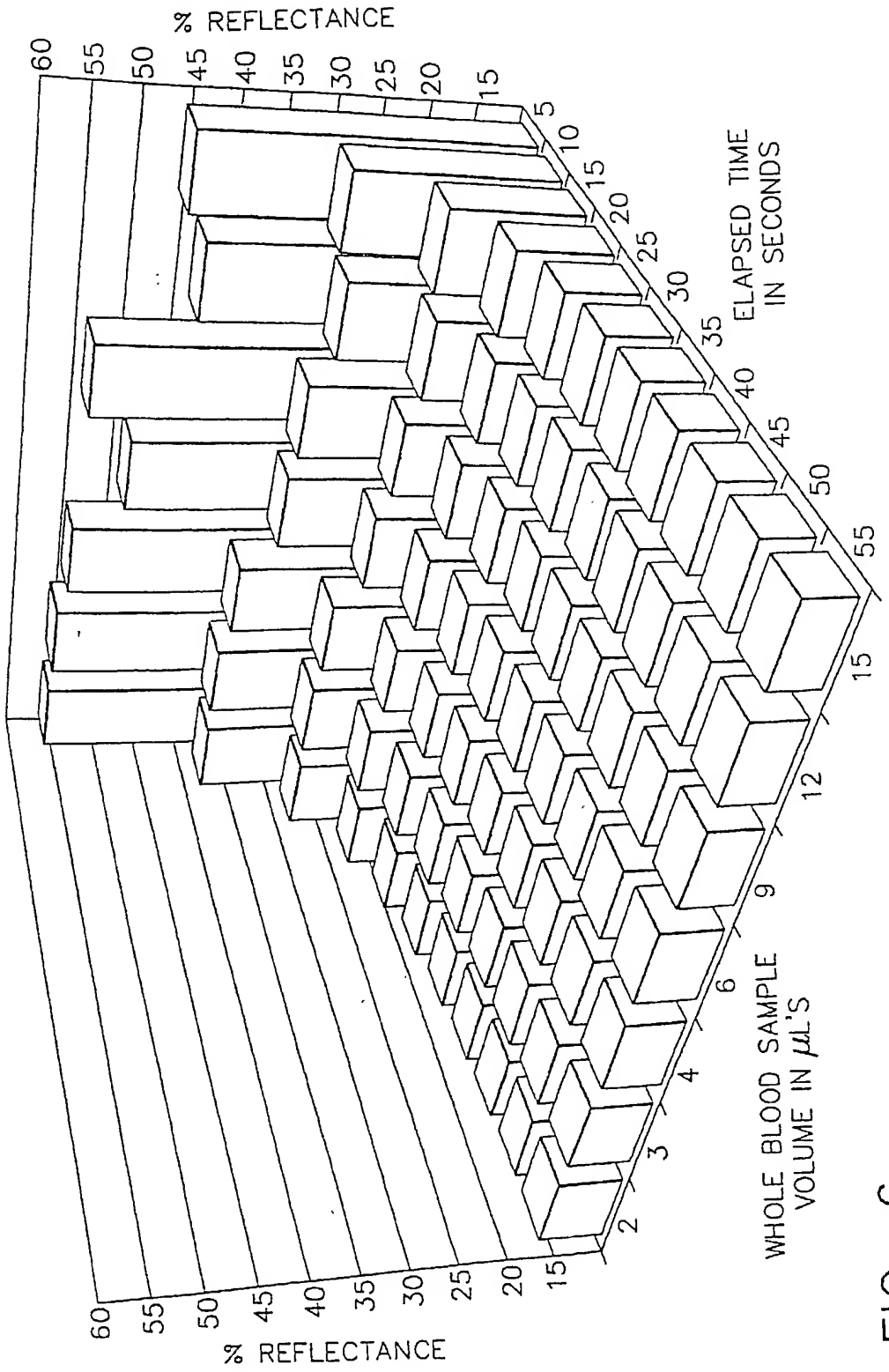
% REFLECTANCE
60
55
50
45
40
35
30
25
20
15
5
10
15
20
25
30
35
40
45
50
55
ELAPSED TIME
IN SECONDS
15
12
9
6
4
3
2
WHOLE BLOOD SAMPLE
VOLUME IN μL'S
60
55
50
45
40
35
30
25
20
15
% REFLECTANCE

FIG. 6

Attorney Docket No. 01726-0001 (PCT)

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if two or more names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Apparatus and Method for Determining Substances in a Body Fluid

the specification for which is attached hereto and was assigned International Application No. PCT/US00/16816. The international application entered the U.S. national stage and has been assigned U.S. Serial No. 10/018,546, and includes a Preliminary Amendment filed December 15, 2001, a copy of which is also attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulation, § 1.56.

I hereby claim priority benefits under Title 35, United States Code, § 119, of the following provisional application: United States Provisional Application Serial Number, 60/139,983, filed June 18, 1999

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

This declaration may be executed in counterparts or separate copies, each of which shall be deemed an original and all of which shall comprise a single document

As a named inventor, I hereby appoint Michael C. Bartol, Regis. No. 44025, as attorney to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Post Office Address: Same as above

Signature of first inventor:_____date_____

2. Full name of second inventor: Sunil Anaokar
Residence: 12111 Misty Way
Indianapolis, IN 46163
USA

Citizenship: USA
Post Office Address: Same as above

Signature of second inventor: Sunil Anaokar date 4/15/02

3. Full name of third inventor: John J. Pasqua
Residence: 1011-D Oak Chase Dr.
Tucker, GA 30084
USA

Citizenship: USA
Post Office Address: Same as above

Signature of third inventor:_____date_____

4. Full name of fourth inventor: Michele J. Crispino
Residence: 3717 Toronto Ct.
Indianapolis, IN 46268
USA

Citizenship: USA
Post Office Address: Same as above

Signature of fourth inventor:_____date_____

1. Full name of first inventor: Joel Mitchen
Residence: 12012 47th Avenue
Pleasant Prairie, WI 53158
USA

Citizenship: USA
Post Office Address: Same as above

Signature of first inventor:_____ date_____

2. Full name of second inventor: Sunil Anaokar
Residence: 12111 Misty Way
Indianapolis, IN 46163
USA

Citizenship: USA
Post Office Address: Same as above

Signature of second inventor:_____ date_____

3. Full name of third inventor: John J. Pasqua
Residence: 1011-D Oak Chase Dr.
Tucker, GA 30084
USA

Citizenship: USA
Post Office Address: Same as above

Signature of third inventor: John J. Pasqua date 5/13/02

4. Full name of fourth inventor: Michele J. Crispino
Residence: 15042 Southwest 104th St.
Apt. 1303
Miami, FL 33196
USA

Citizenship: USA
Post Office Address: Same as above

Signature of fourth inventor:_____ date_____

1. Full name of first inventor: Joel Mitchen
Residence: 12012 47th Avenue
Pleasant Prairie, WI 53158
USA

Citizenship: USA
Post Office Address: Same as above

Signature of first inventor:_____date_____

2. Full name of second inventor: Sunil Anaokar
Residence: 12111 Misty Way
Indianapolis, IN 46163
USA

Citizenship: USA
Post Office Address: Same as above

Signature of second inventor:_____date_____

3. Full name of third inventor: John J. Pasqua
Residence: 1011-D Oak Chase Dr.
Tucker, GA 30084
USA

Citizenship: USA
Post Office Address: Same as above

Signature of third inventor:_____date_____

4. Full name of fourth inventor: Michele J. Crispino
Residence: 15042 Southwest 104th St.
Apt. 1303
Miami, FL 33196
USA

Citizenship: USA
Post Office Address: Same as above

Signature of fourth inventor: M. Crispino date_____

5. Full name of fifth inventor: Terence M. Mc Caffrey
Residence: 1715 Roosevelt St.
Hollywood, FL 33020
USA

FL

SW

citizenship: USA
Post Office Address: Same as above

Signature of fifth inventor: Terence M. McCaffrey date 30 Apr 02

6. Full name of sixth inventor: James M. Connolly
Residence: 8181 Morningside Dr.
Indianapolis, IN 46240
USA

FL

SW

Citizenship: USA
Post Office Address: Same as above

Signature of sixth inventor: _____ date _____

7. Full name of seventh inventor: Hyeon-Sook Lee Zeng
Residence:

_____

_____

USA

Citizenship: USA
Post Office Address: Same as above

Signature of seventh inventor: _____ date _____

5. Full name of fifth inventor: Terrence M. McCaffery
Residence:

_____

_____
USA

Citizenship: USA
Post Office Address: Same as above

Signature of fifth inventor:_____date_____

6. Full name of sixth inventor: James M. Connolly
Residence: 8181 Morningside Dr.
Indianapolis, IN 46240
USA

Citizenship: USA
Post Office Address: Same as above

Signature of sixth inventor:_____date April 25, 2002

7. Full name of seventh inventor: Hyeon-Sook Lee Zeng
Residence:

_____

_____
USA

Citizenship: USA
Post Office Address: Same as above

Signature of seventh inventor:_____date_____

5. Full name of fifth inventor: Terrence M. McCaffery
Residence:

_____

_____

USA

Citizenship: USA
Post Office Address: Same as above

Signature of fifth inventor:_____ date_____

6. Full name of sixth inventor: James M. Connolly
Residence: 8181 Morningside Dr.
Indianapolis, IN 46240
USA

Citizenship: USA
Post Office Address: Same as above

Signature of sixth inventor:_____ date_____

7. Full name of seventh inventor: Hyeon-Sook Lee Zeng
Residence: 10642 Mistflower Way,
Indianapolis, IN 46235.
USA

Citizenship: USA
Post Office Address: Same as above

Signature of seventh inventor: Hyeon-Sook Lee Zeng date 4-19-02